

REMOVAL OF BACTERIA AND BACTERIOPHAGE FROM THE AIR  
BY GLASS FIBER FILTERS

by

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Demands are increasing steadily for highly effective air filtration systems in research laboratories where air must always be relatively free of biological organisms.

High speed photography as well as air sampling has shown that common operations such as pipetting, pouring, and vigorous agitation of tubes containing fluid cultures of bacteria often produce bacterial contamination of the surrounding air and nearby surfaces. Mixing broth cultures with a pipette by alternate suction and blowing, making microscopic slide agglutination tests, autopsy of infected animals, and other bacteriological manipulations also release bacterial aerosols. A survey of laboratory acquired infections in the United States reported in 1951 by Sulkin and Pike (1) tabulates a total of 1,334 infections presumably acquired in the laboratory.

The purpose of the present investigation was to determine the effectiveness of glass fiber filters in removing bacteria and viruses from the air. Advantages of spun glass air filter pads are that they (1) can be decontaminated economically by heat, (2) do not disintegrate when moist, and (3) have a low resistance to air flow.

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The initial studies were conducted on 3 types of commercially available spun glass filter pads. The test organisms used were Serratia indica and Escherichia coli T-3 bacteriophage. S indica is a harmless small bacterial organism about 1 micron in length and one-half micron in thickness. E coli T-3 bacteriophage is also a harmless virus organism, somewhat spherical in shape, which ranges in size from 0.02 to 0.05 microns.

The three types of spun glass material initially tested possessed the nomenclature of 25, 50, and 100 FG: Number 25 FG had a pad thickness of one-half inch and a fiber diameter of 2.75 microns; Number 50 FG was 0.5 inch thick and had a fiber diameter of 1.25 microns; Number 100 FG was 1 inch thick and had a fiber diameter of 2.75 microns. Respective pad resistances to air flow at 20 fpm were 0.12, 0.48, and 0.22 inches of water (Figure 1).

The spun glass pads were installed in a filter unit containing wedge shaped pockets supported in a grid type frame. Each frame could hold one to five pockets. In the standard type frame holding 5 pockets, the total net filtering area was approximately 50 sq. ft. and the normal capacity was 1000 cfm. The pockets were so designed that filtering material from one-half inch to one inch in depth could be placed in them (Figure 2).

Initial tests were conducted on a surface area of 20 sq. ft. with an air volume of 400 cfm, equivalent to 20 fpm through the filter. After the data were assembled and analyzed, it was decided to test a larger filter with a capacity of 2,800 cfm at the same linear flow of 20 fpm. Unless otherwise noted, results refer to the 400 cfm filter.

A Chicago type nebulizer (2) was used to create the aerosol. Biological material from this nebulizer is not always unicellular, because agglomeration of organisms may occur during or after release of the aerosol.

Figure 3 shows the method of air sampling used to determine the bacterial efficiency of the filters. Two sieve type air samplers were placed in the duct work before the filter and two after it. The concentration of organisms before the filter was so adjusted that there were 85 to 240 organisms per cu ft of air. This low concentration was used to approximate the order of magnitude of bacteria in the air under the actual conditions encountered in schools and hospitals, where counts of 23 per cu ft have been reported. Air was drawn through each sampler for three minutes at the rate of 1 cfm. The organisms entering the sieve sampler were impinged on a growth medium of corn steep agar. After incubation at a temperature of 30°C (86°F) for 24 hours, the bacterial colonies were counted. The efficiency of the filter was calculated by comparing the bacterial count of the air before and after filtration. In addition to using low concentrations of S. indica, the filters were tested with much higher concentrations through the use of a cloud of bacteriophage having an average count of  $3.1 \times 10^6$  particles per cu ft.

The results are summarized in Figures 4 and 5.

1. The bacterial arrestance of the 25 FG material varied from 23 to 90 per cent. The average bacterial efficiency was 60 per cent for 23 tests.
2. The arrestance of the 50 FG material was much higher. With the 400 cfm filter and a single half-inch layer of spun glass, the range of efficiency in 20 tests was 88 to 97 per cent, with an average of 94.

When two layers of spun glass were used, the efficiency in 14 tests was 93 to 100 per cent, with an average of 97 per cent. These results indicate considerable variation. Later work indicated that higher and more consistent efficiencies can be expected. The greater early variation

a concentration of  $3.1 \times 10^6$  particles of bacteriophage per cu ft of air in front of the filter. Samples of air were taken before and after the air entered the filter.

Results of counting the bacteriophage particles collected by the air samplers are shown in Figures 5 and 6. The work summarized in Figure 5 represents a 2,800 cfm filter, while Figure 6 represents the results of initial tests on a 400 cfm filter. The 25 FG spun glass filter had an efficiency of only 30 to 38 per cent. One layer of 50 FG spun glass removed 98 to 99 per cent of the organisms, while two layers removed 99 per cent. The 100 FG material rated about 90 per cent.

These studies show that it is possible to set up a spun glass air filtration system having a bacterial and viral filtration efficiency of 99 per cent.

In addition to the use of spun glass as filter material for building ventilation systems, tests were conducted to determine its practicability for use as a filter for bacteriological cabinets, animal cages and shaking machine containers. The readily available supply of spun glass, the low pressure drop across the filter pad, low cost, and the ease with which the spun glass can be installed in a filter frame suggested its use for such laboratory equipment.

A practical compactly arranged spun glass filter unit having a capacity up to 300 cfm and an overall resistance of 2 inches of water was designed, developed and tested (Figure 7). The unit is about 2-1/4 by 3-1/4 ft by 8 inches in size. The results of tests on this filter compared favorably with the original data collected. The weighted average efficiency of the filter for an average S indica concentration of 350 organisms per cu ft

of air was 99.68 per cent, while for a concentration of 69,650 organisms per cu ft the filter efficiency was 99.95. Concentrations of phage particles varying from 30,870 to 298,000 per cu ft of air were removed with an efficiency of 99.74 per cent. It is believed that these efficiencies are sufficient to permit use of this filter in all situations in which air containing minor aerosols incidental to common laboratory techniques is to be filtered and discharged to the air outside a building.

Consideration also had to be given to protective measures required in changing the spun glass filter pads. To avoid exposure of personnel to the contaminated filter, it was desirable to decontaminate the filter in place. Accordingly, the filter unit was designed with strip heaters capable of heating the interior of the filter cabinet to more than 200°C.

Time and temperature studies showed that after the interior of the filter reached 200°C, an exposure of 20 minutes was sufficient to destroy Bacillus globigii spores.

#### USE OF SPUN GLASS IN ANIMAL CAGES

It has been reported in the literature that some pathogenic organisms can be isolated from the feces and urine of infected animals. This would indicate the possibility of airborne infection from animal cages. One way to minimize this danger is to ventilate cages in such a manner that the air is filtered as it enters and leaves. Use of a filter serves several purposes: (1) it protects personnel in the animal room; (2) it lowers the possibility of cross contamination between cages; and (3) in the event of a failure of the mechanical air supply system, contaminated air does not

escape but the animals still receive enough circulation of air through the filters to prevent suffocation. The filter for incoming air consists of a bored rubber stopper with a grooved section into which are fitted a true arc inside retaining ring, a 32 mesh screen, two layers of spun glass, a second 32 mesh screen, and a second true arc ring. (Figure 8) This preliminary model now is under further development. The arrangement provides a tight fit which prevents leakage of organisms between the spun glass and the sides of the rubber stopper. The air leaving the cage may pass through a similar filter or be piped to a larger filter unit.

#### USE OF SPUN GLASS IN A SHAKING MACHINE

The breakage of cultured flasks on a mechanical shaker is a problem which becomes a hazard in laboratories working with pathogenic agents. Loss of plugs from the culture flasks being shaken has also been reported. The possibility of accidentally dropping a flask during transfer to or from the shaker must not be overlooked. To avoid the danger in these possibilities, a shaking machine container has been constructed, which completely encloses the flask yet provides sufficient air through the spun glass filter for satisfactory bacterial growth (Figures 9 and 10).

The shaking machine container is cylindrical and equipped with a spring device which securely holds a 250 or 500 ml flask in place. The head is made of pyrex glass and aluminum. A circular opening in the center of the head houses a spun glass filter held in position by pyrex glass secured in a groove with a true arc retaining ring. Several tests were conducted to determine whether inclosure of a culture flask would affect the growth rate of bacteria. All results indicated that there was no effect upon the growth rate of the bacteria.

NEW DEVELOPMENTS IN FILTER MATERIAL

In the past, a commercially available asbestos and cellulose paper filter has been used. This filter is easily combustible and disintegrates when exposed to water or air saturated with moisture for any considerable time. To overcome these difficulties, several research laboratories and paper mills are developing a glass paper that will be equivalent or better than present air filter papers. One such glass paper possessing glass fibers between 0.2 and 1.5 microns in diameter has been produced commercially by a paper mill and is undergoing adaptation to non-combustible frames. Preliminary tests in our laboratories of the filter material with bacteria showed penetration of 0.01 to 0.001 per cent with most of the data falling near 0.001 per cent. It is evident that it is only a matter of months until there will be available a paper-thin noncombustible filter which is not affected by moisture or chemicals. The glass paper filter will have an efficiency equivalent to or better than that of asbestos paper filters.

It is encouraging to witness the rapid progress that is being made in research laboratories throughout the country as well as by industry in developing high efficiency filters.

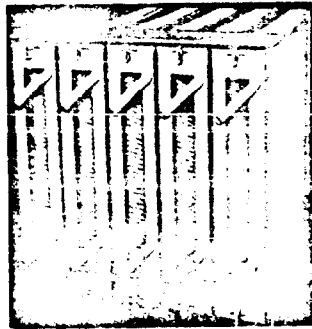
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1. Sulkin, E. S. and Pike, R. M., Survey of Laboratory Acquired Infections, American Journal of Public Health, 44, 769-781.
2. Rosebury, Theodor, Experimental Airborne Infection, 1947, p. 81.



NO. 100 FG	NO. 50 FG	NO. 25 FG
THICKNESS 1"	0.5"	0.5"
FIBER DIA. 2.75	1.25	2.75

Fig. 1—Spun glass filter material.



Deep bed filter unit—air-entering side—showing spring latches that hold pockets securely in frame against heavy sponge rubber seals to prevent air leakage.

Fig. 2—Spun glass air filter unit front view.

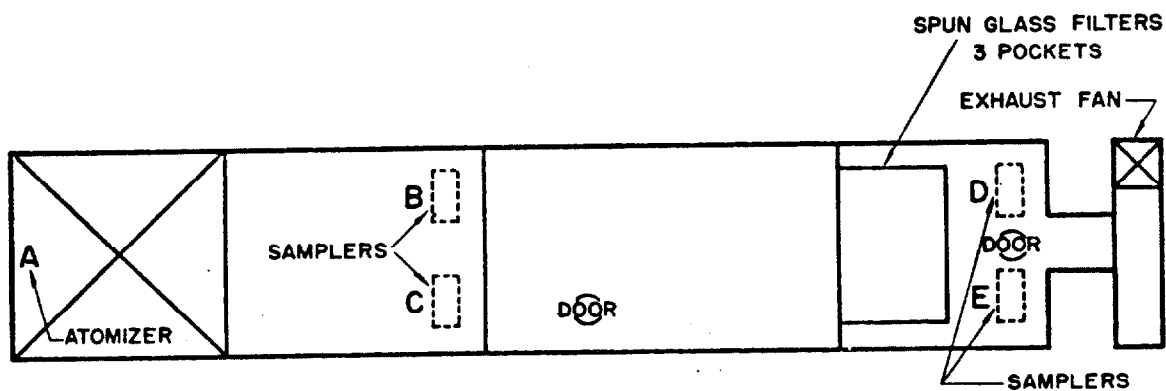


Fig. 3—Air filtration test unit.



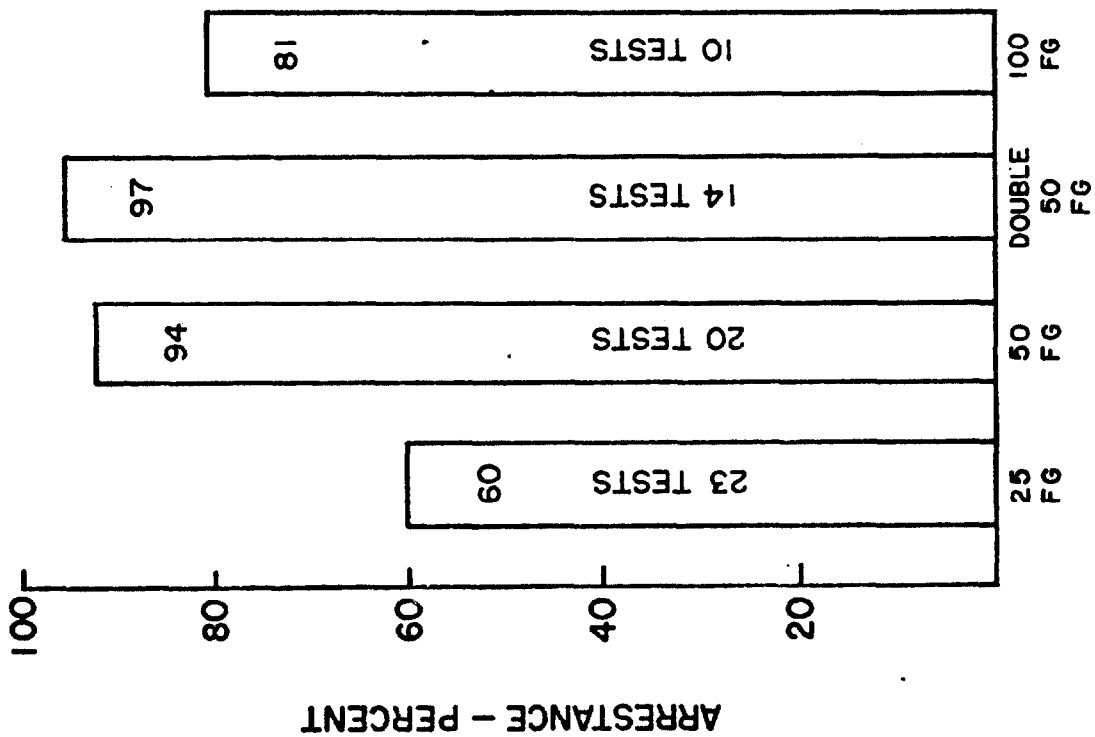


Fig. 4—Comparison of average arrestances when removing serratia indica with spun glass filters (400 cfm filter).

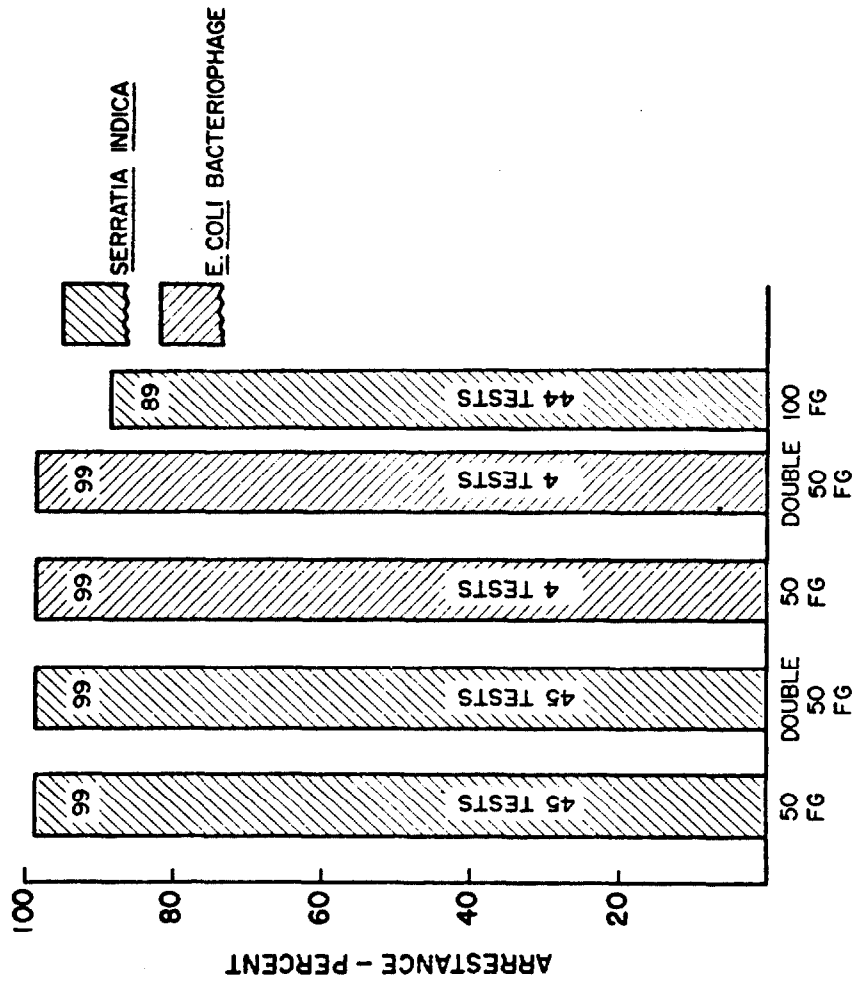


Fig. 5—Comparison of average arrestances obtained in removing serratia indica and *E. coli* bacteriophage T-3 with spun glass filters (2800 cfm filter).

TYPE OF FILTER	NUMBER OF TESTS	EFFICIENCY-%	
		TESTED BY LIQUID IMPINGER	TESTED BY SIEVE SAMPLER
25-FG SPUN GLASS	1	30	38
50-FG SPUN GLASS	1	98	99
50-FG + 50-FG	1	99	99
100-FG SPUN GLASS	1	90	90

Fig. 6—Approximate efficiency of various filters removing E. coli bacteriophage T-3 from the air.



Fig. 7 — Air filter for bacteriological safety cabinets.

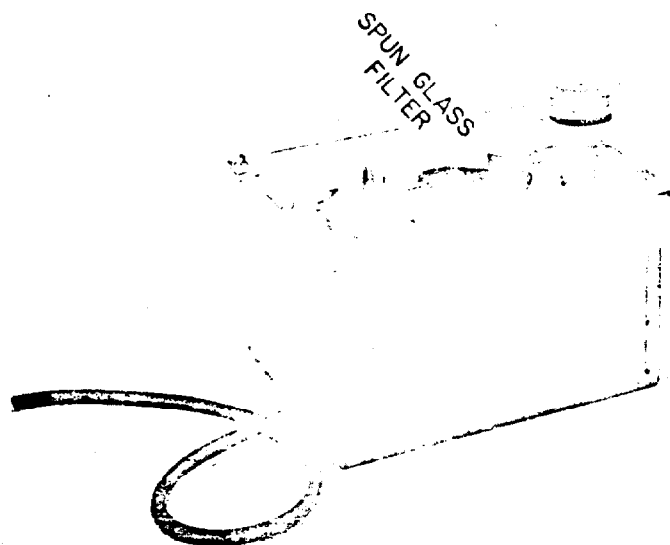


Fig. 8—Mechanically ventilated animal cage.

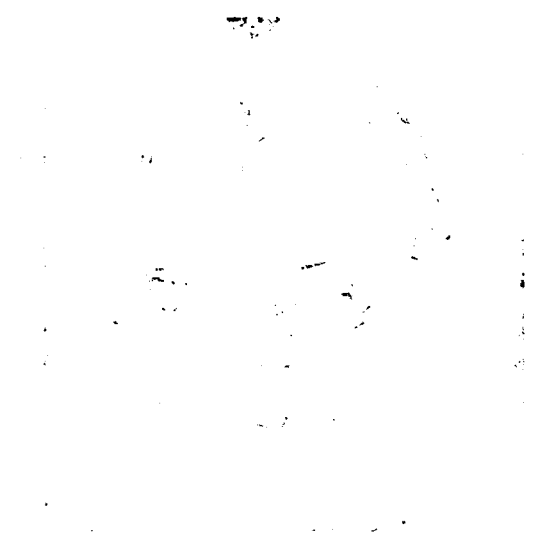


Fig. 9—Shaking machine container.

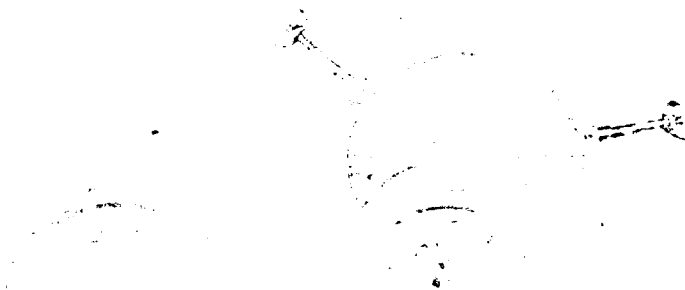


Fig. 10—Container with spun glass air inlet for shaking machine flasks.